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Persistent organic pollutants (POPs) in human milk: A biomonitoring study in rural areas of Flanders (Belgium)

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HIGHLIGHTS

- POP levels were measured in the human milk samples of 84 mothers.
- The concentrations were compared to the Belgian WHO human milk study (2006).
- For most pollutants lower or comparable concentrations were found.
- While for ΣDDT and metabolites, *trans*-nonachlor and HBCD higher levels were observed.
- Associations were found between POP levels and personal characteristics/dietary habits.

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ABSTRACT

To collect information on the concentrations of persistent organic pollutants (POPs) in the rural areas in Flanders (Belgium), 84 breastfeeding mothers were recruited in rural communities in East and West Flanders and Flemish Brabant in 2009–2010. Polychlorinated biphenyl (PCB) congeners, organochlorine pesticides, brominated flame retardants, perfluorinated compounds, polychlorinated dibenzodioxines and dibenzofurans, and dioxin-like PCBs were measured in individual milk samples and in a pooled milk sample, while some additional pollutants were only measured in the pooled sample. For most pollutants, the concentrations in this study were lower or comparable to the concentrations measured in the pooled Belgian sample of the WHO human milk study of 2006, except for the pesticides dichlorodiphenyltrichloroethane DDT (+25% for ΣDDT and metabolites) and *trans*-nonachlor (+94%), and for the brominated flame retardant hexachlorocyclododecane HBCD (+153%). Perfluorinated compounds were for the first time determined in human milk samples from Belgium and the concentrations were comparable to those from other European countries. Also, interesting associations were found between the concentrations of POPs measured in human milk and personal characteristics as well as dietary habits of the study population. PFOS en PFOA concentrations were significantly higher in milk of primiparous participants compared to mothers who gave birth to their second child. Lower brominated PBDE congeners increased with increasing BMI of the mothers ($p = 0.01$ for BDE 47, $p = 0.02$ for BDE 99 and $p = 0.02$ for BDE 100). Participants consuming milk or dairy products daily had significant higher concentrations of ΣDDTs ($p = 0.03$) and oxychlordane ($p = 0.047$) in their human milk samples.

1. Introduction

In the first Flemish Environment and Health survey, performed by the Flemish Centre of Expertise on Environment and Health

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(FLEHS I, 2002–2006), internal human exposure to pollutants in areas differing in pollution pressure was measured with selected biomarkers. A selected number of biomarkers of exposure (cadmium, lead, PCDD/Fs, PCBs, DDE – a metabolite of the pesticide DDT, HCB, 1-hydroxypyrene – a general marker of polyaromatic hydrocarbon exposure, and t,t′-muconic acid – a marker of exposure to benzene) and of effect (e.g. growth and development, fertility, asthma and allergy, and genotoxicity) were analyzed in 14–15 year-old adolescents (n = 1679), in adults between 50 and 65 years of age (n = 1583) and in mother–child pairs (n = 1196). In all age groups, participants living in low populated rural communities of East and West Flanders and Flemish Brabant showed significantly higher body burdens of PCBs, dioxin-like substances and chlorinated pesticides (DDE and HCB) compared to other, more densely populated Flemish regions (Schroijen et al., 2008; Koppen et al., 2009). Due to the health concern associated with increased body burdens of chlorinated POPs, a follow-up study of the concentrations of pollutants in these rural areas was undertaken. A newborn cohort was chosen for this study, since newborns are more vulnerable to adverse effects of toxic exposures and since early life exposures could then be linked to the later development of these infants. Because of the lipophilic nature of POPs, human milk was chosen to assess exposure to POPs. Human milk is a valuable matrix for human biomonitoring of lipophilic pollutants, since it is a non-invasive sample that is available in sufficient quantities (e.g. compared to cord blood). However, human milk samples also have some disadvantages: only breastfeeding mothers can participate and the occurrence of non-lipophilic pollutants can be low. On the other hand, cord blood can be sampled from all mothers, but the sampling procedure is more laborious for the maternities and some pollutants cannot be measured (e.g. pollutants that cannot cross the placenta or that are only present in very low concentrations in the blood, like HBCD). A further advantage of human milk was the possibility to compare with results from the previous WHO-coordinated human milk study in Belgium (2005–2006). Therefore, POPs were not only measured in individual human milk samples but also in a pooled sample (composed from all individual milk samples) by the WHO reference laboratory. The aims of this study were (1) to investigate if the selected POPs could be easily measured in human milk samples (i.e. are they above LOQ or not), (2) to compare the concentrations measured in human milk samples from the rural area to the concentrations measured in milk samples from the Belgian WHO-coordinated study from 2006 and (3) to determine the associations between POP concentrations and personal characteristics (age of the mother, BMI, etc.) and dietary habits.

2. Materials and methods

2.1. Selection and recruitment of participants

The participants were recruited via nine maternities in 24 low-populated rural communities in East and West Flanders and Flemish Brabant (Fig. 1). The study area was selected according to the criteria set in the FLEHS I study (Schroijen et al., 2008). Since the POP concentrations were meant to be compared with the Belgian results of the 4th WHO-coordinated human milk survey from 2006, the selected mothers were thought to meet the WHO inclusion criteria with respect to age and parity, and had to be living for at least 5 years in the study area (Colles et al., 2008). However, since recruitment of the participants went slow, the selection criteria were slightly modified. The participants had to be 18–35 years old breastfeeding women, giving birth to a first or second child (no twins) after a normal pregnancy (>36 weeks). The mothers had to be born in Belgium and had to reside in one of the selected sampling areas for at least 5 years. Both mother and child had to be in a healthy condition and HIV negative. An informed consent had to be signed by the mothers prior to enrolment. The study design was approved by the medical–ethical committee of the University of Antwerp on 10th of July 2009.

The mothers were asked to complete a questionnaire for information about residence during the last 5 years, date and place of birth, mother's age, weight and length, dietary habits and occupation, smoking and alcohol consumption, fertility and health data, exposure to pollutants indoor or in the workplace, social economical status and perception of environmental problems.

During the recruitment period of 14 months (May 2009 until end of June 2010), 284 breastfeeding mothers between 18 and 35 years old and giving birth to a first of second child were approached. However, 41.3% of the selected mothers did not live for 5 years in the study area, 14.9% refused participation and 10.3% did not deliver a milk sample. Finally, a total of 84 mothers (30.6%) participated in the study.

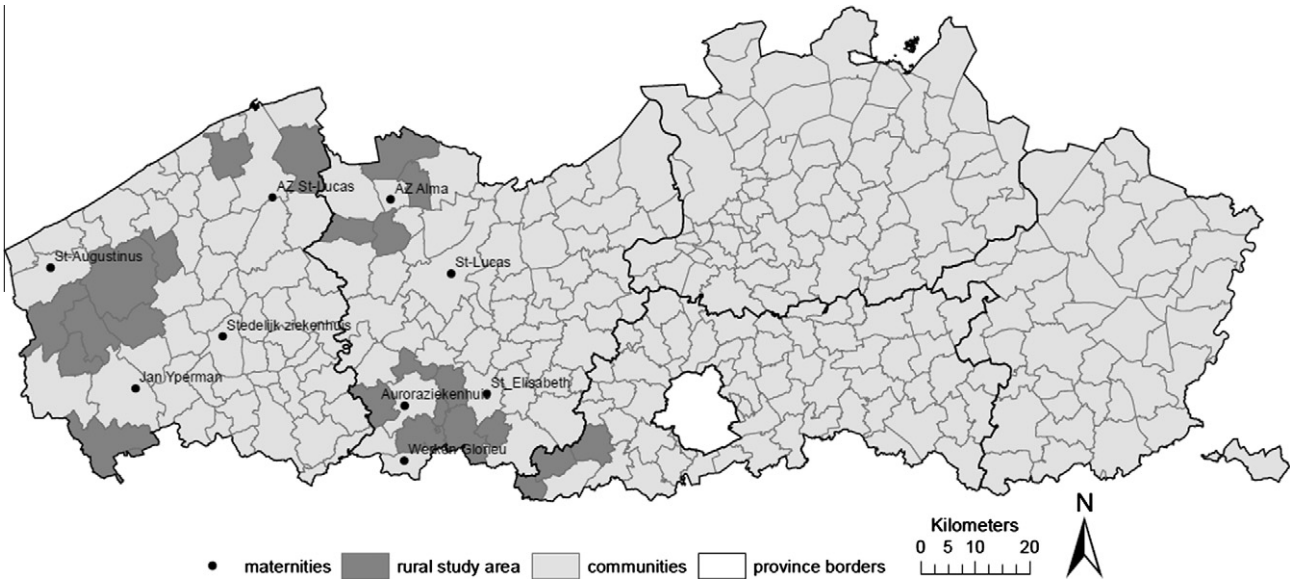

Fig. 1. Overview of the selected rural communities and maternities for recruitment of the participants in the rural areas of Flanders (Belgium).

2.2. Sample collection

Between 2 and 8 weeks after delivery, the newborn mothers collected the breast milk at their homes. A manual human milk pump (AVENT type ISIS Naturally) and a polypropylene bottle (Cellstar®) were provided at the first contact with the mothers in the maternity within the week after delivery. At least 50 mL of milk samples needed to be collected, after and/or during nursing. Afterwards, the bottle was stored in the refrigerator (+4 °C) for maximum 72 h and then in the freezer (⩽18 °C). Fieldworkers visited the mothers at their homes to collect the samples. The samples were stored at -20 °C until analysis.

2.3. Analytical procedures

In all 84 individual samples PCB congeners, organochlorine pesticides (*p,p′*-DDE and *p,p′*-DDT, HCB, β- en γ-HCH, *trans*-nonachlor and oxychlordane), brominated flame retardants (PBDEs), PCDD/Fs and dioxin-like PCBs were measured, while perfluorinated compounds were only measured in a subset of 40 individual samples. From each of the 84 individual samples, 10 mL human milk of the initial sample was taken to compose a pooled sample. This pooled sample was used to follow the time trend in all pollutants that were measured during the 4th WHO-coordinated human milk campaign in 2006 (Colles et al., 2008). GC–HRMS analysis of pollutants in the pooled sample was done by the WHO reference lab (State Institute for Chemical and Veterinary Analysis of Food (CVUA), Freiburg, Germany).

PCB congeners, organochlorine pesticides and PBDE congeners were determined by the Toxicological Centre at the University of Antwerp (UA, Belgium). The method was based on the protocols described by Covaci and Schepens (2001), Covaci and Voorspoels (2005) and Covaci et al. (2001). Briefly, human milk samples (5 mL) were spiked with internal standard solutions (PCB 143, ε-HCH, BDE 77, BDE 128 and ^{13}C-BDE-209) and afterwards extracted using solid phase extraction (SPE) on an OASIS HLB (6 mL, 500 mg) cartridge, before purification on an acid silica column. The final extract was analyzed by GC–MS. For PCBs, HCB, *p,p′*-DDE and *p,p′*-DDT a HT-8 column (25 m × 0.22 mm × 0.25 μm) with the MS in electron impact ionization (EI) mode was used, while for the analysis of PBDEs and other pesticides, a DB-5 column (15 m × 0.25 mm × 0.10 μm) was used with the MS in electron capture negative ionization (ECNI) mode. The quality control (QC) was done by regular analysis of procedural blanks (n = 8), replicate samples (one replicate for each 20 samples) and CRM 450 (PCBs in powdered milk, n = 2). For the replicate samples and CRM 450, a RSD < 10% for the deviation from the mean or certified values, respectively, was considered acceptable. Limits of quantification (LOQ) varied between 0.4 and 2.0 ng/g lipid weight for PCBs and OCPs and between 0.08 and 0.8 ng/g lipid weight for PBDEs. The method performance was demonstrated by successful participation in international inter-laboratory exercises organized by NIST and AMAP on the determination of PBDEs, OCPs and PCBs in biota. Accuracy and recoveries for individual analytes were between 85% and 105% (RSD < 15%).

Perfluorinated compounds (PFCs) were analyzed at the National Institute of Public Health, (Oslo, Norway). Human milk samples were prepared and analyzed for seven PFCs (PFHxS, PFHpS, PFOS, PFOA, PFNA, PFDA and PFUnDA) using isotope dilution, column-switching LC–MS/MS (Haug et al., 2009) as described by Thomsen et al. (2010a). Procedural blanks (n = 3), analyzed together with the samples, did not contain any of the PFCs above limit of quantification (LOQ). For quantification of PFOS, the total area of the linear and branched isomers was integrated. High quality of the determinations was assured by analyzing in triplicate, two samples from a proficiency test on determination of PFCs in breast milk (n = 3 + 3). The obtained concentrations were within ±1 SD of the consensus value for all PFCs found above the LOQ (B. van Bavel, personal communication).

PCDD/Fs and dioxin-like PCBs were analyzed with the CALUX bioassay at the Department of Analytical and Environmental Chemistry at the Free University of Brussels (VUB, Brussels, Belgium). The method was based on the protocol described by (Croes et al., 2011), with some small modifications to account for the higher fat content of the human milk samples. Briefly, human milk samples were extracted with a hexane/acetone mixture and filtered upon a pre-conditioned Celite column. After extraction, the amount of fat was weighted and the extracts were cleaned up on a pre-conditioned multi-layer silica column coupled in series with a carbon column, with separation of the PCDD/Fs and dl-PCBs. Both fractions were re-dissolved in a defined volume hexane (1.5 mL and 2 mL for the dl-PCB and PCDD/F fractions, respectively) to be dosed the CALUX bioassay. CALUX analysis of both fractions was done with the sensitive H1L7.5c1 mouse hepatoma cell line (Denison et al., 2008; He et al., 2011). Cell treatment and measurement were performed according to the EPA method 4435 as described by Croes et al. (2011). Procedural blanks (n = 4), QC standards (n = 19) and reference milk samples (n = 10) were analyzed together with the samples.

2.4. Statistics

Means, medians, percentiles and geometric means (after ln transformation) were calculated for all measured compounds using Statistica 8.0. To determine the factors that influence the POP concentrations in the human milk samples, first, univariate regression relationships were calculated for the compounds measured in the individual samples. Covariates with a p-value lower than 0.20 were included in a multiple regression analysis. Covariates strongly correlating (r > 0.70) were not included together in the model. For all statistical calculations, the POP concentrations were expressed per unit of volume (except for the PCDD/Fs and dl-PCBs, which were expressed in pg BEQ/g lipid for comparison reasons) and for samples below the limit of quantification (LOQ), half of the LOQ was used. Age and BMI were always included as confounding factors in all models. Milk lipid percentage was included as confounding factor for the PBDEs, PCBs and organochlorine pesticides. For PCBs, pesticides, PCDD/Fs and dl-PCBs, smoking habits were also added in the regression model.

3. Results and discussion

3.1. Short description of the study population

Characteristics of the mothers participating in this survey (number and percentage of participants or mean with minimum–maximum range) are summarized in Table SM1 (Supplementary Material). Initially, the same inclusion criteria as in the WHO-coordinated human milk surveys were used in the present study: max 30 years old, born in Belgium, normal pregnancy, delivery at 36 weeks or later, first born child, no twins. Since the selected regions were lowly populated to Flemish standards, difficulties with recruitment of the participants occurred. Therefore, the inclusion criteria needed to be broadened, which resulted in differences in some of the characteristics of this population compared to the population investigated during the WHO-coordinated study in 2006. The mean age of this study population was 28.5 years (range 20.6–35.6 years old) compared to 26 years in the Belgian WHO 2006 population. 30% of the mothers were older than 30 years (which was the maximum age in the WHO human milk study), while only 17% were younger than 26 years. The mean Body Mass Index (BMI)

(23.0 kg cm^{-2}, range 17.7–45.6 kg cm^{-2}) and the number of mothers being breastfed as newborn (48%) were comparable to the WHO study from 2006. Because of difficulties in recruiting participants in this rural study, mothers who gave birth to their second child, were also enrolled, resulting in less participants having their first child (57%) compared to the Belgian WHO population (100%).

There were also differences in dietary habits between the two study populations. The mothers from the rural areas in this study consumed more fish (high consumption: 18% in this study versus 6% in the WHO study), more meat (80% of the mothers consumed every day meat versus 66% in the WHO study) and more local eggs (57% versus 47%). Especially the consumption of marine fish was higher in the present study (74% versus 38% in the Belgian population of the WHO study) (Colles et al., 2008). These differences can be (at least partially) due to the differences in geographic repartition between the two studies. In the WHO study, mothers residing in whole Belgium (Flanders, Brussels and Wallonia) were recruited and also urban areas with a higher population density were selected. Moreover, the rural areas are located closer to the North Sea, which could explain the higher fish consumption in the present study.

3.2. Concentrations of POPs in the Flemish human milk samples and comparison with international studies

In Table 1, an overview of the results of the POP concentrations, measured in the individual milk samples and in the pooled milk sample, is given. PCB congeners PCB 118, PCB 138, PCB 153, PCB 170 and PCB 180, pesticides HCB, *p,p′*-DDE, oxychlordane and *β*-HCH, dl- PCBs and PCDD/Fs were quantifiable in all 84 individual samples and in the pooled sample. PCB congeners PCB 31, PCB 52, PCB 95 and PCB 149 and the pesticides *α*-chlordane, *γ*-chlordane and *α*-HCH were below LOQ in all individual samples.

When comparing the two pools (this study and WHO 2006 study) of human milk, lower concentrations were seen for most of the pollutants in this study, except for the pesticides DDT (and metabolites) and *trans*-nonachlor. Although the use of both pesticides was banned in Belgium more than 30 years ago, the concentrations of the sum of DDT and its metabolites and of trans-nonachlor in the pooled sample from this study were respectively 25% and 94% higher compared to the results from the WHO-study conducted four years earlier. To investigate if these higher concentrations were due to recent exposure, the pesticide/metabolite ratio for the pooled samples was calculated for both compounds (Table 2). For both DDT and *trans*-nonachlor, the ratios were significantly lower than one, which indicates a historical exposure to these pollutants. The ratio *trans*-nonachlor/oxychlordane was however higher in the rural area compared to the WHO-2006 study, which indicates that the participants were more recently exposed to this pesticide compared to the mothers from the Belgian WHO study in 2006. This more recent exposure could be related to the different dietary habits in the rural study population compared to the WHO study (2006). For example, a higher consumption of lo-

Table 1
Concentration levels (ng g^{-1} lipid) of selected POPs, measured in both the individual samples and in the pooled human milk sample. Comparison with Belgian pooled human milk sample from the WHO 2006 study.

Compound	Concentration (ng g^{-1} lipid)						
	Individual samples of rural area (N = 84)					Pooled samples	
	P_{10}	P_{50}	P_{90}	LOQ (ng L^{-1})	% > LOQ	Rural area (pool of n = 84)	Belgium WHO 2006 (pool of n = 197)
Lipid content (%)	2.30	4.40	6.06	–	–	4.02	3.20
PCB 28	<LOQ	<LOQ	1.161	0.02	41.7	0.88	1.02
PCB 52	<LOQ	<LOQ	<LOQ	0.02	0	0.23	0.36
PCB 101	<LOQ	<LOQ	0.6	0.02	21.4	0.39	0.50
PCB 105	0.2	0.7	1.6	0.02	73.8	1.53	1.82
PCB 118	1.7	3.7	6.7	0.02	100	6.65	9.05
PCB 138	6.7	13.5	23	0.02	100	18.7	21.9
PCB 153	8.1	16.8	30.9	0.02	100	32.8	38.4
PCB 170	2.4	5.2	9.9	0.02	100	na	na
PCB 180	4.3	8.8	17.0	0.02	100	17.2	18.4
Sum 3 PCBs[a]	18.5	38.3	72.4	–	100	68.7	78.7
Sum 6 PCBs[b]	20.2	39.7	73.0	–	100	70.2	80.1
Sum 7 PCBs[c]	22.7	44.0	79.4	–	100	76.9	89.1
HCB	3.6	6.4	9.6	0.02	100	9.6	15.0
Sum DDTs[d]						196	156
p,p′-DDE	23.3	56.9	152	0.05	100	162	132
p,p′-DDT	0.5	2.6	10.9	0.05	72.6	11.0	8.8
Oxychlordane	1.3	2.8	4.9	0.02	100	5.6	8.0
Trans-nonachlor	0.7	1.4	2.8	0.02	95.2	3.3	1.7
β-HCH	3.5	6.1	11.4	0.02	100	8.9	12.0
γ-HCH	<LOQ	<LOQ	0.8	0.01	26.2	nd	0.7
BDE 28	<LOQ	<LOQ	0.07	2	14.3	0.05	0.07
BDE 47	0.07	0.16	0.66	2	97.6	0.67	0.89
BDE 99	0.02	0.06	0.16	2	63.1	0.62	0.22
BDE 100	0.02	0.06	0.21	2	60.7	0.16	0.21
BDE 154	0.03	0.07	0.15	2	79.8	0.02	0.02
BDE 153	0.14	0.29	0.57	2	97.6	0.47	0.49
BDE 183	<LOQ	<LOQ	0.11	3	15.5	0.04	0.05
BDE 209	0.22	0.65	1.84	20	57.1	na	na
dl-PCBs[e]	0.95	1.71	2.89	0.69	100	5.9	7.0
PCDD/Fs[e]	6.09	10.1	18.6	1.15	100	8.4	10.3

[a] Sum 3 marker PCBs = PCB138 + PCB153 + PCB180.
[b] Sum 6 marker PCBs = PCB28 + PCB52 + PCB101 + PCB138 + PCB153 + PCB180.
[c] Sum 7 marker PCBs = PCB28 + PCB52 + PCB101 + PCB118 + PCB138 + PCB153 + PCB180.
[d] Sum DDTs = *o,p′*-DDT, *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD.
[e] The dl-PCBs and the PCDD/Fs were expressed in pg CALUX-BEQ g^{-1} lipid for the individual samples and in pg WHO-TEQ g^{-1} lipid (TEF 1998) in the pooled sample. na is not analyzed; nd is not detected.

Table 2
comparison of the pesticide/metabolite ratios in the pooled sample of the rural area (this study) and in the Belgian sample from WHO survey from 2006.

Ratio of compounds	2009–2010 rural area	2005–2006 Belgium (WHO)
DDT/DDE	0.07	0.07
Trans-nonachlor/oxychlordane	0.59	0.21

cal food products and/or fish could lead to a higher intake rate of these POPs in the rural area.

The perfluorinated compounds PFOS and PFOA were detectable in the whole subset of 40 samples, while PFNA and PFHxS were only quantifiable in 42.5% and 20% of the samples, respectively. The compounds PFDA, PFUnA and PFHpS were in all samples below LOQ (Table 3). These compounds were measured for the first time in Belgian human milk samples. Perfluorinated alkyl compounds are used in a great variety of consumer products and industrial processes and have been found in human samples worldwide. Concentrations of PFOS in human milk in this study were in line with the mean breast milk concentrations found in Germany (0.12 ng mL^{-1}, 2006) (Volkel et al., 2008). A study from Hungary (1996) reported PFOS median concentration of 0.33 ng mL^{-1} (Volkel et al., 2008), while in Sweden (2004) median PFOS concentrations of 0.20 ng mL^{-1} were found (Kärrman et al., 2007). In a recent study in Spain (2007–2008), PFOS and PFHxS were the only PFCs detected in human milk, with mean concentrations of 0.12 and 0.04 ng mL^{-1}, respectively (Kärrman et al., 2010). Tao et al. (2008) found mean PFOS, PFOA, PFHXS and PFNA concentrations of 0.13, 0.44, 0.15 and 0.07 ng mL^{-1} in human milk samples collected in the USA in 2004. Other perfluorinated compounds (PFDA, PFUnA, PFHpA, PFDoDA, PFBS) were in more than 90% of the samples below the detection limit in these USA samples.

Table 4 shows the concentration concentrations of POPs that were only measured in the pooled milk samples. Results from both the pooled samples from the rural area and the Belgian WHO study from 2006 were compared to evaluate the spatial (rural area of Flanders versus Belgium) and temporal trends. Both pooled samples were analyzed by the same WHO reference laboratory (CVUA, Freiburg, Germany). The pesticides aldrin, endrin, endrin keton, heptachlor and *trans*-heptachlor epoxide, some DDT metabolites (*o,p′*-DDD, *o,p′*-DDE, *o,p′*-DDT), toxaphene congener Parlar 62, Mirex, octochlorodipropyl ether (S-421), endosulfans (endosulfan-sulphate, alpha- and beta-endosulfan), α-chlordane, γ-chlordane, α-HCH, bromocyclen, 4,4′-methoxychlor, nitrophen and pendimethalin could neither be detected in the pooled milk samples from 2006 (Colles et al., 2008) nor in the present human milk pool confirming the observation that they are not of concern for the Belgian population.

Since synthetic musk fragrances are present in most cosmetics and personal care products (like aftershaves, soaps, lotions) and have half-life times in humans around 3 months (Reiner et al., 2007), the concentrations found in the human milk will probably depend on the frequency and quantity of the use of these products (Roosens et al., 2007). Musk xylene, mostly used as synthetic substitute of the natural musks in detergents and soaps, could not be detected in the pooled sample from this campaign, while in the WHO study from 2006 a concentration of 11.7 ng g^{-1} lipid was found for this compound. Musk keton, also a synthetic nitromusk mostly applied in cosmetics, was found at about the same low concentrations as in 2006. Since the use of both compounds in cosmetics and personal care products has been restricted in the EU since 2004, nitromusks are more and more replaced by less persistent polycyclic aromatic musks, like HHCB and AHTN (Hu et al., 2010; Yin et al., 2012). It is thus expected that the body burden of nitromusks in the human body will further decrease.

The concentrations of nitromusks in Belgian milk were rather low compared to other European and non-European studies. For examples, in a USA human milk survey of 2004, mean concentrations of 30.0 ng g^{-1} lipid and 74.5 ng g^{-1} lipid were found for musk xylene and musk keton, respectively (Reiner et al., 2007) while in China mean concentrations of 24 ng g^{-1} lipid for muks xylene and 9 ng g^{-1} lipid musk keton were found (Zhang et al., 2011). Duedahl-Olesen et al. (2005) observed mean concentrations of 23.6 ng g^{-1} lipid for musk xylene and 17 ng g^{-1} lipid for musk keton in the milk of mothers residing in Denmark.

All other pollutants, measured in the pools (Tables 1 and 4), were in the same range or lower in the rural area compared to the Belgian WHO-coordinated study from 2006, except for HBCD, which was 153% higher in the current study (Table 4). These higher HBCD concentrations could be due to the restrictions on the use of PBDEs in the European Union (Thomsen et al., 2010b). Due to the possible health risks, the production of all PDBEs, with exception of deca-BDE, for electronic equipment was forbidden in the European Union in 2006 (Directive, 2002/95/EC). Since September 2010, also production of deca-BDE for electronic equipment was no longer allowed within the EU (Commission Decision, 2010/571/EU). However, as HBCD is mainly used as flame retardant in expanded polystyrene (EPS) used for insulation purposes of buildings, one could speculate that increased HBCD exposure could also result from increased use of EPS in constructions of buildings to reduce energy costs. Another explanation is that the rural population is exposed to HBCD through the diet. A recent publication showed that HBCD concentrations in Belgian fish samples were elevated (Roosens et al., 2008). The concentration of HBCD in the human milk samples from this study (3.8 ng g^{-1} lipid) was higher than the mean concentrations found in other European studies. In two Swedish studies from 2002 and 2004 (Lignell et al., 2003, 2005) and in a French study from 2005 (Antignac et al., 2008), HBCD concentrations of respectively 0.35, 0.33 and below LOQ were found. In a recent Spanish study from 2006, higher HBCD concentrations (median concentration of 27 ng g^{-1} lipid) were detected in human milk (Eljarrat et al., 2009), while in Norway, much lower mean HBCD concentrations of 0.86 ng g^{-1} lipid were found (Thomsen et al., 2010b).

3.3. Determinants of POP concentrations in human milk

Using multiple regression analysis, a statistical significant positive association with the participants' age (divided in classes) was only obtained for β-HCH ($p = 0.02$) after adjustment for the confounders BMI, smoking and lipid content. An age dependent in-

Table 3
overview of the concentrations (ng mL^{-1}) of the perfluorinated compounds, measured in a subset of 40 individual milk samples. nc is not calculated, because of low levels. PFDA, PFUnA, PFHpS were below the LOQ in all 40 samples.

Compound (ng mL^{-1})	Mean	95% CI	P_{10}	P_{50}	P_{90}	LOQ (ng mL^{-1})	% > LOQ
PFOS	0.13	0.09–0.13	0.07	0.10	0.22	0.01	100
PFOA	0.08	0.06–0.09	0.06	0.07	0.15	0.01	100
PFNA	nc	nc	<LOQ	<LOQ	0.02	0.01	42.5
PFHXS	nc	nc	<LOQ	<LOQ	0.02	0.01	20

Table 4
Comparison of the concentration levels of additionally measured POPs in the pooled human milk samples of the rural area (this study) and the Belgian sample of the WHO 2006 human milk survey. The concentration levels are expressed in ng g^{-1} lipid. nd = not detected.

Compound (ng g^{-1} lipid)	2009–2010 rural area	2005–2006 Belgium (WHO)
Aldrin	nd	nd
Dieldrin	7.2	6.7
Endrin group		
Endrin	nd	nd
Endrin keton	nd	nd
Heptachlor group		
Heptachlor	nd	nd
Cis-heptachlor-epoxide	4.7	5.6
Trans-heptachlor-epoxide	nd	nd
Parlar (toxaphenes)		
Parlar 26	0.5	0.7
Parlar 50	1.8	1.5
Parlar 62	nd	nd
Mirex	nd	nd
S-421	nd	nd
Muks xylene	nd	11.7
Muks keton	1.7	1.9
Hexabromocyclododecane (HBCD)		
Sum HBCDs	3.80	1.50
α-HBCD	3.20	1.50
β-HBCD	0.05	<0.08
γ-HBCD	0.55	<0.08

crease in POP concentrations, like β-HCH, can be expected since this compound accumulates in the human body. This relationship was found in many studies and also in the serum of adults studied during the Flemish human biomonitoring programs (Den Hond et al., 2009). PFOS en PFOA concentrations were significantly higher in milk of primiparous participants compared to mothers who gave birth to their second child ($p = 0.01$ for PFOS and $p = 0.03$ for PFOA) and significant lower values for PFOS were observed in participants living 5 to 20 years in the study area compared to mothers residing in the area for more than 20 years ($p = 0.007$). This was also described by Tao et al. (2008) who measured significantly lower PFOS (−49%) and decreased PFOA (−32%), PFHxS (−14%) and PFNA (−30%) concentrations in milk from multiparous women compared to primiparous participants.

The tetra- and penta-BDE congeners increased with increasing BMI of the mothers ($p = 0.01$ for BDE 47, $p = 0.02$ for BDE 99 and $p = 0.02$ for BDE 100). This was also reported for human milk samples from the USA (Daniels et al., 2010) and Germany (Hoopmann et al., 2012). For most POPs (e.g. PCBs) lower concentrations are expected with higher a BMI, but much less is known about PBDEs and concentrations measured in human milk could be different compared to other tissues. A possible explanation for these findings between PBDEs in human milk and BMI could be that the lower chlorinated PBDEs are metabolized or excreted slower. Another correlation was found between PCDD/Fs and weight change after pregnancy. Participants who lost weight after the pregnancy relative to their weight before pregnancy had significant higher values of PCDD/Fs compared to participants who gained weight ($p = 0.003$). Weight losses result in a decrease of adipose tissue and thus in a release of POPs into blood and human milk. Significant relations were also found between POP concentrations and dietary habits. Participants daily consuming milk or dairy products had significant higher concentrations of DDT ($p = 0.03$) and oxychlordane ($p = 0.047$) in their breast milk samples. Samples of participants not eating vegetables from their own garden, contained significant lower HCB concentrations ($p = 0.03$). HCB is a pesticide that was often used in gardens as a fungicide. Since 1974 the production and use of this compound is forbidden in Belgium, but HCB can still come into the environment as a by-product of chemical industry or due to waste incineration. If this compound is present in the soil, it can be taken up by plants such as cucumbers, pumpkins, carrots, and radishes. (Hilber et al., 2008; Waliszewski et al., 2008; Mikes et al., 2009). HCB can therefore be present in higher concentrations in the human milk through the consumption of vegetables from the own garden. Similarly, analysis of cord blood samples in the same area during FLEHS I (2003–2004) had also indicated a weak but significant positive relationship between consumption of local dairy/animal fat and concentrations of PCBs, *p,p′*-DDE and CALUX-BEQ in cord blood (Koppen et al., 2009).

4. Conclusions

In this study (rural areas in Flanders, Belgium; 2009–2010), the concentrations of most POPs measured in the pooled human milk sample were lower than in the Belgian sample of the WHO-coordinated study, which was held 4 years earlier. However, some pollutants like metabolites of the pesticide DDT, the pesticide *trans*-nonachlor and the brominated flame retardant HBCD were higher in the present study. The higher concentrations of chlorinated pesticides found in human milk of participants residing in this rural area confirm the results from the FLEHS I study where higher POP concentrations were observed in the (cord)blood samples of adolescents, adults and newborns. Since the use of these chlorinated pesticides is banned in Belgium for more than 30 years and low pesticide/metabolite ratios were found in the present study, it can be concluded that the higher body burdens are due to historical exposure. It is thus expected that the concentrations in the rural area of Flanders will further decrease over time. HBCD on the other hand showed the highest increase in concentrations compared to the former WHO study and is thus a pollutant of concern. Although it was later introduced into the market and the environment and restrictions on its use through REACH are already implemented, it is important to monitor if concentrations of this pollutant will further increase in the rural areas and in the Flemish population in general.

Human milk seems to be the most interesting matrix to investigate time trends of HBCD concentrations in newborns, since this compound could only be detected in 0.8% of the cord blood samples measured during FLEHS II (2007–2011, unpublished results). Other POPs, e.g. PCBs, PCDD/Fs, HCB, DDT and metabolites, PBDEs and musks, could be quantified in cord blood samples (FLEHS II) and in human milk samples from this study. Choice of the matrix should thus depend on the pollutants of interest. Cord blood and human milk can also give complementary information, since human milk reflects the mothers' body burden of lipophilic compounds, while cord blood shows the concentrations of pollutants of the foetus.

Another important issue in this study was the selection of the participants. Due to the low number of primiparous breastfeeding women in the age category of the WHO criteria, the selection criteria had to be broadened. To be able to fulfill the WHO inclusion criteria and to facilitate the comparison of new data with other national and international studies, selection of a larger, dense populated study area with a larger number of births, or a longer recruitment period, would be advisable for future studies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.06.058.

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